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1. Your reference — **P034643GB: CJM**

26 JUN 2003 ✓

2. Patent application number *(The Patent Office will fill in this part)* — 0315021.6 ✓

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

CHIRON SRL
VIA FIORENTINA 1
53100 SIENA
ITALY

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — **ITALY**

8608812001

4. Title of the invention — **IMMUNOGENIC GONOCOCCAL COMPOSITIONS**

5. Name of your agent *(if you have one)*

Carpmaels & Ransford

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

43 Bloomsbury Square
London
WC1A 2RA

Patents ADP number *(if you know it)* — **83001**

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

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11. I/We request the grant of a patent on the basis of this application.

Signature: Carpmaels & Ransford — Date: 26th June 2003

12. Name and daytime telephone number of person to contact in the United Kingdom: **CAMERON J. MARSHALL** **020-7242 8692**

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IMMUNOGENIC GONOCOCCAL COMPOSITIONS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Neisseria gonorrhoeae* (gonococcus) and their use in immunisation.

BACKGROUND ART

N.gonorrhoeae is a bacterial pathogen which causes diseases including gonorrhoea, urethritis, cervicitis and pelvic inflammatory disease. In addition, like other inflammatory STDs, infection is believed to enhance HIV transmission.

N.gonorrhoeae is related to *N.meningitidis* (meningococcus). Sequence data are now available for serogroup B of meningococcus {*e.g.* refs. 1 to 6} and also for serogroup A {7}. It is a further object of the invention to provide proteins and nucleic acid useful in distinguishing between gonococcus and meningococcus and, in particular, between gonococcus and serogroup B meningococcus.

Various gonococcal antigens have been described {*e.g.* ref. 8}, but there is currently no effective vaccine against *N.gonorrhoeae* infection. It is an object of the invention to provide materials useful in vaccine development.

Vaccines against pathogens such as hepatitis B virus, diphtheria and tetanus typically contain a single protein antigen (*e.g.* the HBV surface antigen, or a tetanus toxoid). In contrast, acellular whooping cough vaccines typically have at least three *B.pertussis* proteins, and the Prevenar™ pneumococcal vaccine contains seven separate conjugated saccharide antigens. Other vaccines such as cellular pertussis vaccines, the measles vaccine, the inactivated polio vaccine (IPV) and meningococcal OMV vaccines are by their very nature complex mixtures of a large number of antigens. Whether protection against can be elicited by a single antigen, a small number of defined antigens, or a complex mixture of undefined antigens, therefore depends on the pathogen in question.

It is an object of the invention to provide further and improved compositions for providing immunity against gonococcal disease and/or infection. The compositions are based on a combination of two or more gonococcal antigens.

DISCLOSURE OF THE INVENTION

Within the many proteins of the gonococcal genome, six have been found to be particularly suitable for immunisation purposes, particularly when used in combinations. The invention therefore provides a composition comprising two or more of the following antigens: (1) OmpA; (2) OmpH; (3) PPIase; (4) ngs41; (5) ngs117; and (6) App. These are referred to herein as the 'six basic antigens'.

The composition may comprise three or more, four or more, five or more, or all six of the six basic antigens. Preferred compositions comprise: (1) OmpA & OmpH; (2) OmpA & PPIase; (3) OmpA & ngs41; (4) OmpA & ngs117; (5) OmpA & App; (6) OmpH & PPIase; (7) OmpH & ngs41; (8) OmpH & ngs117; (9) OmpH & App; (10) PPIase & ngs41; (11) PPIase & ngs117; (12) PPIase & App; (13) ngs41 & ngs117; (14) ngs41 & App; and (15) ngs117 & App.

(1) OmpA protein

The 'OmpA' protein has been disclosed as SEQ ID NO[s]: 25 & 26 in reference 8 (SEQ ID NO: 2 herein).

Preferred OmpA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 2; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID 2, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 2. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 2. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 45 or more) from the N-terminus of SEQ ID NO: 2. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The transmembrane domain of ngs13 (numbered relative to SEQ ID NO: 1) is at around residues 36-52.

The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

(2) OmpH protein

The sequence of 'OmpH' protein in gonococcal strain FA1090 is SEQ ID NO: 3 herein (see also SEQ ID NO[s]: 6055 & 6056 of reference 8).

Preferred OmpH proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID 3, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpH proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably at least 19) from the N-terminus of SEQ ID NO: 3. Other fragments omit one or more domains of the protein (*e.g.*

omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain *e.g.* residues 20-36 of SEQ ID NO:3, or of an extracellular domain).

Residues 74-129 may form a coiled-coil domain, and so the OmpH protein may be present in the form of an oligomer *e.g.* a dimer, trimer, tetramer, *etc.*

(3) Peptidyl-prolyl cis/trans isomerase (PPIase) protein

The 'PPIase' protein has been disclosed as part of SEQ ID NO[s]: 1033 & 1034 in reference 8 (SEQ ID NO: 4 herein).

Preferred PPIase proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 4; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID 4, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PPIase proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 4. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 4. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 4. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

(4) Ngs41 protein

The 'Ngs41' protein has been disclosed as SEQ ID NO[s]: 81 & 82 in reference 8 (SEQ ID NO: 5 herein).

Preferred Ngs41 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID 5, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Ngs41 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 5. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 5. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 5. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(5) Ngs117 protein

The 'Ngs117' protein has been disclosed as SEQ ID NO[s]: 233 & 234 in reference 8 (SEQ ID NO: 6 herein).

Preferred Ngs117 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 6; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID 6, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Ngs117 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 6. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 6. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 6. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(6) App

The gonococcal 'App' protein has been disclosed as SEQ ID NO[s]: 653 & 654 in reference 1, and as SEQ ID NO[S]: 1087 & 1088 in reference 8 (SEQ ID NO: 7 herein). It is related to the meningococcal adhesion penetration protein (App) disclosed in reference 9.

Preferred App proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID 7, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These App proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 7. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 7. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 7. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The App protein is subject to autoproteolysis, and so a proteolytic fragment of SEQ ID NO: 7 may be used.

Fusion proteins

The six basic antigens may be present in the composition as six separate polypeptides, but it is preferred that at least two (*i.e.* 2, 3, 4, 5 or 6) of the antigens are expressed as a single polypeptide chain (a 'hybrid' polypeptide) *e.g.* such that the six antigens form fewer than six polypeptides. Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or

poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

A hybrid polypeptide included in a composition of the invention may comprise two or more (*i.e.* 2, 3, 4, 5, 6) of the six basic antigens. Hybrids consisting of two or three of the six basic antigens are preferred.

Within the combination of six basic antigens, an antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Two-antigen hybrids for use in the invention comprise: (1) OmpA & OmpH; (2) OmpA & PPIase; (3) OmpA & ngs41; (4) OmpA & ngs117; (5) OmpA & App; (6) OmpH & PPIase; (7) OmpH & ngs41; (8) OmpH & ngs117; (9) OmpH & App; (10) PPIase & ngs41; (11) PPIase & ngs117; (12) PPIase & App; (13) ngs41 & ngs117; (14) ngs41 & App; and (15) ngs117 & App.

Hybrid polypeptides can be represented by the formula NH_2-A-{-X-L-}$_n$-B-COOH, wherein: X is an amino acid sequence of one of the six basic antigens as defined above; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of {-X-L-}, linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more); and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID 1), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18,

17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, n is 2 or 3.

The invention also provides nucleic acid encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions, non-glycosylated, lapidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other neisserial or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other neisserial or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Strains

Preferred polypeptides of the invention comprise an amino acid sequence found in gonococcal strain FA1090.

Where hybrid polypeptides are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Heterologous host

Whilst expression of the polypeptides of the invention may take place in gonococcus, the invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeasts, *etc.*

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of two or more (*e.g.* 3, 4, 5, 6) of the six basic antigens in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is

preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a gonococcus (*e.g.* gonorrhoea, urethritis, cervicitis and pelvic inflammatory disease, *etc.*).

One way of checking efficacy of therapeutic treatment involves monitoring gonococcal infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the six basic antigens after administration of the composition.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal, topical, transdermal {*e.g.* see ref. 10} or transcutaneous {*e.g.* see refs. 11 & 12}, intranasal {*e.g.* see ref. 13}, ocular, aural, pulmonary or other mucosal administration.

The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

Gonococcal infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference 14.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Preferred further adjuvants include, but are not limited to: (A) aluminium salts, including hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphoshpates, orthophosphates), sulphates, *etc.* {*e.g.* see chapters 8 & 9 of ref. 15}), or mixtures of different aluminium compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred; (B) MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer) {see Chapter 10 of 15; see also ref. 16}; (C) liposomes {see Chapters 13 and 14 of ref. 15}; (D) ISCOMs {see Chapter 23 of ref. 15}, which may be devoid of additional detergent {17}; (E) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion {see Chapter 12 of ref. 15}; (F) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (G) saponin adjuvants, such as QuilA or QS21 {see Chapter 22 of ref. 15}, also known as Stimulon™ {18}; (H) chitosan {*e.g.* 19}; (I) complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA); (J) cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating

factor, tumor necrosis factor, *etc.* {see Chapters 27 & 28 of ref. 15}; (K) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) {*e.g.* chapter 21 of ref. 15}; (L) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions {20}; (M) a polyoxyethylene ether or a polyoxyethylene ester {21}; (N) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol {22} or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol {23}; (N) a particle of metal salt {24}; (O) a saponin and an oil-in-water emulsion {25}; (P) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) {26}; (Q) *E.coli* heat-labile enterotoxin ("LT"), or detoxified mutants thereof, such as the K63 or R72 mutants {*e.g.* Chapter 5 of ref. 27}; (R) cholera toxin ("CT"), or detoxified mutants thereof {*e.g.* Chapter 5 of ref. 27}; (S) double-stranded RNA; (T) microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) being preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB); (U) oligonucleotides comprising CpG motifs *i.e.* containing at least one CG dinucleotide; (V) monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 {28}; (W) polyphosphazene (PCPP); (X) a bioadhesive {29} such as esterified hyaluronic acid microspheres {30} or a mucoadhesive selected from the group consisting of cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose; or (Y) other substances that act as immunostimulating agents to enhance the effectiveness of the composition {*e.g.* see Chapter 7 of ref. 15}. Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant toxins are preferred mucosal adjuvants.

Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

The composition may include an antibiotic.

Further antigens

The composition contains six basic antigens. It may also include further antigens, although it can contain no gonococcal protein antigens other than the six basic antigens. Further antigens for inclusion may be, for example:

- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 31 from serogroup C {see also ref. 32} or the oligosaccharides of ref. 33.
- antigens from *Helicobacter pylori* such as CagA {34 to 37}, VacA {38, 39}, NAP {40, 41, 42}, HopX {*e.g.* 43}, HopY {*e.g.* 43} and/or urease.
- a saccharide antigen from *Streptococcus pneumoniae* {*e.g.* 44, 45, 46}.

- a protein antigen from *Streptococcus pneumoniae* {*e.g.* 47}.
- an antigen from hepatitis A virus, such as inactivated virus {*e.g.* 48, 49}.
- an antigen from hepatitis B virus, such as the surface and/or core antigens {*e.g.* 49, 50}.
- an antigen from hepatitis C virus {*e.g.* 51}.
- a diphtheria antigen, such as a diphtheria toxoid {*e.g.* chapter 3 of ref. 52} *e.g.* the CRM_{197} mutant {*e.g.* 53}.
- a tetanus antigen, such as a tetanus toxoid {*e.g.* chapter 4 of ref. 52}.
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 {*e.g.* refs. 54 & 55}; whole-cell pertussis antigen may also be used.
- a saccharide antigen from *Haemophilus influenzae* B {*e.g.* 32}.
- polio antigen(s) {*e.g.* 56, 57} such as OPV or, preferably, IPV.
- a protein antigen from *N.meningitidis* serogroup B {*e.g.* refs. 1-6 & 58-63}
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 64, 65, 66, 67, *etc.*
- an antigen from *Chlamydia trachomatis* {*e.g.* 68}.
- an antigen from *Chlamydia pneumoniae* {*e.g.* refs. 69 to 75}.
- an antigen from *Porphyromonas gingivalis* {*e.g.* 76}.
- an antigen from *Treponema pallidum*.
- rabies antigen(s) {*e.g.* 77} such as lyophilised inactivated virus {*e.g.* 78, RabAvert™}.
- measles, mumps and/or rubella antigens {*e.g.* chapters 9, 10 & 11 of ref. 52}.
- influenza antigen(s) {*e.g.* chapter 19 of ref. 52}, such as the haemagglutinin and/or neuraminidase surface proteins.
- antigen(s) from a paramyxovirus such as respiratory syncytial virus (RSV {79, 80}) and/or parainfluenza virus (PIV3 {81}).
- an antigen from *Moraxella catarrhalis* {*e.g.* 82}.
- an antigen from *Streptococcus pyogenes* (group A streptococcus) {*e.g.* 83, 84, 85}.
- an antigen from *Streptococcus agalactiae* (group B streptococcus) {*e.g.* 86}.
- an antigen from *Staphylococcus aureus* {*e.g.* 87}.
- an antigen from *Bacillus anthracis* {*e.g.* 88, 89, 90}.
- a papillomavirus antigen *e.g.* from any HPV type.
- a herpes simplex virus antigen *e.g.* from HSV-1 or HSV-2.
- an antigen from a virus in the flaviviridae family (genus flavivirus), such as from yellow fever virus, Japanese encephalitis virus, four serotypes of Dengue viruses, tick-borne encephalitis virus, West Nile virus.
- an antigen from a HIV *e.g.* a HIV-1 or HIV-2.
- an antigen from a rotavirus.

- a pestivirus antigen, such as from classical porcine fever virus, bovine viral diarrhoea virus, and/or border disease virus.
- a parvovirus antigen *e.g.* from parvovirus B19.
- a coronavirus antigen *e.g.* from the SARS coronoavirus.
- a prion protein (*e.g.* the CJD prion protein)
- an amyloid protein, such as a beta peptide {91}
- a cancer antigen, such as those listed in Table 1 of ref. 92 or in tables 3 & 4 of ref. 93.

The composition may comprise one or more of these further antigens. The composition may include at least one further bacterial antigen and/or at least one further viral antigen. It is preferred that combinations of antigens should be based on shared characteristics *e.g.* antigens associated with respiratory diseases, antigens associated with enteric diseases, antigens associated with sexually-transmitted diseases, *etc.*

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {*e.g.* refs. 94 to 103}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred {104}. Other carrier polypeptides include the *N.meningitidis* outer membrane protein {105}, synthetic peptides {106, 107}, heat shock proteins {108, 109}, pertussis proteins {110, 111}, protein D from *H.influenzae* {112}, cytokines {113}, lymphokines {113}, hormones {113}, growth factors {113}, toxin A or B from *C.difficile* {114}, iron-uptake proteins {115}, *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means {55}.

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used {*e.g.* refs. 116 to 124}. Protein components of the compositions of the

invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 125. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in reference 126.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows western blotting using anti-OmpA serum. Figure 2 shows similar data for anti-OmpH serum. Figure 3 shows anti-OmpH western blot data for a variety of clinical isolates.

Figures 4 and 6 show expression of PPIase in extracts of cell culture over time. Figure 5 shows anti-PPIase western blot data for a variety of clinical isolates. Figure 6 shows

Figure 7 shows a western blot of whole cells using anti-App serum. App is seen in the intact cells of two strains (lanes 1 & 3) but not in the isogenic knockout mutants (lanes 2 & 4). Figure 8 is a western blot showing App expression over time. Figures 9 and 10 show FACS analysis of App expression.

MODES FOR CARRYING OUT THE INVENTION

The six basic antigens

The six antigens OmpA, OmpH, PPIase, ngs41, ngs117 and App were individually expressed in *E.coli* and purified. Antibodies against the six proteins were made in mice, and the antibodies were used for western blots against gonococcus F62, to detect cell surface expression.

The OmpA protein could be seen in gonococcus using the anti-OmpA serum (Figure 1, lanes 1 & 4). It could also be seen in OMVs prepared from gonococcus (lanes 3 & 6). In isogenic deletion mutants, however, no immunoreactive band could be seen (lanes 2 & 5)

The OmpH protein was detected in gonococcus by the anti-OmpH sera (Figure 2, lanes 1 & 3). In isogenic knockout mutants of gonococcus, however, no immunoreactive band was visible (Figure 2,

lanes 2 & 4). Expression of OmpH across various clinical isolates was also tested by western blot. As shown in Figure 3, immunoreactive bands were seen in isolates from Baltimore USA (top left), from the UK (bottom left) and from Korea (top right).

Autotransporters, such as App, are synthesised as large precursor proteins comprising at least three functional domains: the N-terminal leader sequence, the passenger domain, and the C-terminal domain (β-domain). The leader sequence mediates the export of the protein in to the periplasm, the β-domain inserts into the outer membrane and allows the export of the passenger domain. Once at the bacterial surface, the passenger domain can be cleaved and released in the environment. The expression data for gonococcal App was consistent with this model – full-length protein was seen on the cell surface of F62 and FA1090 strains by western blot (Figure 7, showing full-length ~160kDa protein and also cleavage products; see also Figure 8, lanes 1-4) and by FACS (Figure 9), was seen by western blot on the surface of OMVs prepared from log-phase cells (Figure 8, lane 5), was found by western blot to be processed and secreted in the culture supernatant (Figure 8, lanes 6-9), , but no protein was detected when using isogenic knockouts either by western blot (Figure 7) or by FACS (Figure 9). In addition, a C3 binding assay showed that App is able to elicit antibodies which activate the complement cascade (Figure 10).

Adhesion studies

The role of the six basic antigens in gonococcal adhesion was studied using knockout strains. The ability of wild-type and knockout strains to bind to and then invade ME-180 (epithelial-like human cells from cervical carcinoma) or Hec1B (epithelial-like human cells from endometrial adenocarcinoma) cells was compared.

Adhesion assays were performed using the epithelial cells seeded in 96-well tissue-culture plates and grown in Medium 199 with the addition of 10% FCS, until confluency. Gonococci grown on GC agar were suspended in Dulbecco's complete phosphate-buffered saline (PBSB) and used to infect cell monolayers at 200-100 bacteria/cell. At the end of a 3-hour incubation at 37°C in 5% CO_2 (v/v), total colony-forming units (cfu) were estimated after addition of 1% saponin to the wells. Adhesiveness was quantified by determining the ratio of cell-associated cfu/total cfu present in the assay.

For invasion experiments, intracellular bacteria were recovered after treatment for 2 hours with gentamicin (200μg/ml), to kill extracellular bacteria. Results were presented as ratio of the adhesiveness of the tested strain to that of the high-adhesive control.

OmpH knockouts showed a 7-fold reduction in adhesion and a 12-fold reduction in invasion. Ngs13 knockouts showed a 2-fold reduction in adhesion and a 5-fold reduction in invasion. PPIase knockouts showed a 30-fold reduction in adhesion and a similar reduction in invasion. App knockouts showed a 2-fold reduction in adhesion and a 5-fold reduction in invasion.

PPIase

SEQ ID NO: 4 shows sequence identity to macrophage infecivity potentiator (MIP) from *Legionella pneumophila*, which is a PPIase. The PPIase activity of the gonococcal protein has been confirmed by an *in vitro* assay on a purified recombinant protein comprising SEQ ID NO: 4 and a C-teminus histidine tag.

In the F62 strain, PPIase protein is detected in the total cell extracts as time progresses. The protein is secreted in the culture supernatant during growth (Figure 4) and is present in the outer membrame vesicles (OMV) indicating a surface-localization.

PPIase is present in total extracts obtained from all clinical isolates analysed (10 from Baltimore, 7 from Korea and 4 from England). The positive and negative control are the strain F62 and the relative isogenic mutant Δ576 (Figure 5).

The PPIase gene was cloned in the expression vector pET under the T7 promoter and expressed in *E.coli* BL21(DE3) strain. After 1 hour of IPTG induction (Figure 6, left panel) the protein is detected in total extract (t) and in soluble fraction (s). The protein is progressively secreted in the culture supernatant (Figure 6, Sn in right panel).

The ability of gonococci to survive intracellularly in the RAW264 cell line was assessed for wild-type and Δ576. The number of intracellular bacteria was determined after 30 min, 1h and 3h of infection followed by gentamicin treatment. In the knockout strain there is a reduction of 3-10 fold of intracellular survival.

Adhesion and invasion assays showed that the Δ576 knockout mutant was less effective.

Combinations

After expression and purification, the six antigens were combined in pairs, triples, quadruples, *etc.* The efficacy of the combined antigens was tested in a mouse model of *N.gonorrhoeae* infection and was compared to the efficacy of the antigens alone, and also against adjuvant-only controls. The antigens (single and combined) were administered to the mice in combination with various adjuvants.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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SEQUENCE LISTING

SEQ ID NO: 1

GSGGGG

SEQ ID NO: 2

MTFFKPSTVVLTASALALSGCVADPVTGQQSPNKSAMYGLGGAAVCGIVGALTHSGKGARNSALACGAIGAGVGGYMDYQEQRLRQNL
AGTQIEIQRQGNQIRLVMPESVTFATGSAALGGSAQYALNTAAQTLVQYPDTTLTINGHTDNTGSDAVNNPLSQHRAQAVAYYLQTRG
VAASRLTVYGYGSHMPVASNATVEGRAQNRRVEILINPDQRAVNAARHM

SEQ ID NO: 3

MPSEALQTAFRGNIRRSFTMIRLTRAFAAALIGLCCTTGAHADTFQKIGFINTERIYLESKQARNIQKTLDGEFSARQDELQKLQREG
LDLERQLAGGKLKDAKKAQAEEKWRGLVEAFRKKQAQFEEDYNLRRNEEFASLQQNANRVIVKIAKQEGYDVILQDVIYVNTQYDVTD
SVIKEMNAR

SEQ ID NO: 4

MNTIFKISALTLSAALALSACGKKEAAPASASEPAAASAAQGDTSSIGSTMQQASYAMGVDIGRSLKQMKEQGAEIDLKVFTDAMQAV
YDGKEIKMTEEQAQEVMMKFLQEQQAKAVEKHKADAKANKEKGEAFLKENAAKDGVKTTASGLQYKITKQGEGKQPTKDDIVTVEYEG
RLIDGTVFDSSKANGGPATFPLSQVIPGWTEGVRLLKEGGEATFYIPSNLAYREQGAGEKIGPNATLVFDVKLVKIGAPENAPAKQPD
QVDIKKVN

SEQ ID NO: 5

MILASLVRYYRRLATETDETGNPKVPSYGFSEEKIGWILVLDKEGRLKTVVPNLTADKKPQPKLMSVPRPEKRTSGIKPNFLWDKTAY
ALGVEANKNKAEAKEKPFTPSEKTFEAFKQYHLDLLQNSEDEGLQALCRFLQNWQPAHFAAENLPAEMLDSNTAFSLEKPTALIHKRE
AAQTLWAGCLKSDEALESLCLISGDTAPIARLHPAIKGVFGGQSSGGSIISFNKEAFSSFGKEQGANAPVSEQSAFAYTTALNYLLRR
ENNHCLTIGDASTVFWAEADDIVD

SEQ ID NO: 6

MVAYAFLFLFVTAAVLLIVRSHYRWTYFFASALFVFLAGGMLMLTAQWQRALNFASVWFVVLILFHRLKIHYYKQPLLISDFLLIADW
RNWETLFHYKEAVIGMAGLLALAGYAVFGWSGADSLGMPWRWAGAVLFAAAFVSVRHFSKHPGAVKTWLDSLPDDGRDVFLNLPMSCR
AVFFQVPVFEGDGEAFARQMPSETRPYGMSDEKPDIVVTLMESTLDPHCFDFAAAKIPDLKMFGRQEDTVFSSPLRVHTFGGATWKSE
FAFLAGVPSTDFGALASGVFYSVVPHLQTGFVRNLREHGYFCVALSPFTKGNYNAKAAYDHFGFNLMFQPQDLGYPAPMGKNLWHISS
EEMMQYARMILEKRHPDLENVRQPMFVYVLTMKEHGPYRTDTDNVFDLDAPDLNAKTVSALNDYIGRIADLDKAVESFDRYLHERGKP
FVFGYFGDHQVPFEGVSVRKKWDYAQPDYVTQFAVRSNIAGGFVQRQDFLDLAFAGGVLMEAAGLEAKDGFMRANMAMRGLCGGGLED
CPNRELVGNYRNYLYDVLKIAR

SEQ ID NO: 7

MKTTDKRTTETHRKAPKTGRIRFSPAYLAICLSFGILPQARAGHTYFGINYQYYRDFAENKGKFAVGAKDIEVYNKKGELVGKSMTKA
PMIDFSVVSRNGVAALAGDQYIVSVAHNGGYNNVDFGAEGSNPDQHRFSYQIVKRNNYKAGTNGHPYGGDYHMPRLHKFVTDAEPVEM
TSYMDGWKYADLNKYPDRVRIGAGRQYWRSDEDEPNNRESSYHIASAYSWLVGGNTFAQNGSGGGTVNLGSEKIKHSPYGFLPTGGSF
GDSGSPMFIYDAQKQKWLINGVLQTGNPYIGKSNGFQLVRKDWFYDEIFAGDTHSVFYEPHQNGKYFFNDNNNGAGKIDAKHKHYSLP
YRLKTRTVQLFNVSLSETAREPVYHAAGGVNSYRPRLNNGENISFIDKGKGELILTSNINQGAGGLYFEGNFTVSPKNNETWQGAGVH
ISDGSTVTWKVNGVANDRLSKIGKGTLLVQAKGENQGSVSVGDGKVILDQQADDQGKKQAFSEIGLVSGRGTVQLNADNQFNPDKLYF
GFRGGRLDLNGHSLSFHRIQNTDEGAMIVNHNQDKESTVTITGNKDITTTGNNNNLDSKKEIAYNGWFGEKDATKTNGGLNLNYPPEE
ADRTLLLSGGTNLNGNITQTNGKLFFSGRPTPHAYNHLGSGWSKMEGIPQGEIVWDNDWIDRTFKAENFHIQGGQAVVSRNVAKVEGD
WHLSNHAQAVFGVAPHQSHTICTRSDWTGLTSCTEKTITDDKVIASLSKTDVRGNVSLADHAHLNLTGLATFNGNLVQAETRTIRLRA
NATQNGNLSLVGNAQATFNQATLNGNTSASDNASFNLSNNAVQNGSLTLSDNAKANVSHSALNGNVSLADKAVFHFENSRFTGKISGG
KDTALHLKDSEWTLPSGTELGNLNLDNATITLNSAYRHDAAGAQTGSAADAPRRRSRRSLLSVTPPTSAESRFNTLTVNGKLNGQGTF
RFMSELFGYRSGKLKLAESSEGTYTLAVNNTGNEPVSLEQLTVVEGKDNTPLSENLNFTLQNEHVDAGAWRYQLIRKDGEFRLHNPVK
EQELSDKLGKAGETEAALTAKQAQLAAKQQAEKDNAQSLDALIAAGRNATEKAESVAEPARQAGGENAGIMQAEEEKKRVQADKDTAL
AKQREAETRPATTAFPRARRARRDLPQPQPQPQPQPQRDLISRYANSGLSEFSATLNSVFAVQDELDRVFAEDRRNAVWTSGIRDTKH
YRSQDFRAYRQQTDLRQIGMQKNLGSGRVGILFSHNRTGNTFDDGIGNSARLAHGAVFGQYGIGRFDIGISAGAGFSSGSLSDGIRGK
IRRRVLHYGIQARYRAGFGGFGIEPHIGATRYFVQKADYRYENVNIATPGLAFNRYRAGIKADYSFKPAQHISITPYLSLSYTDAASG
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FIGURE 1

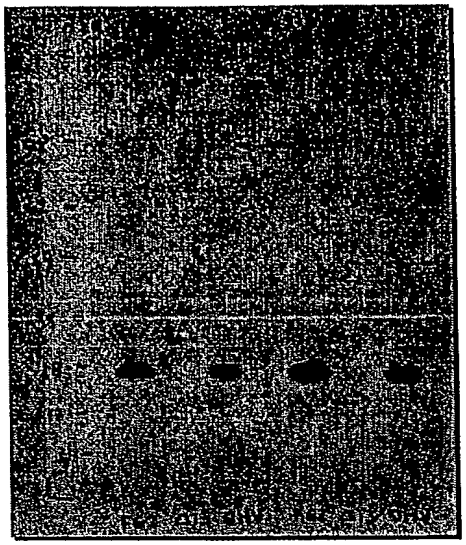


FIGURE 2

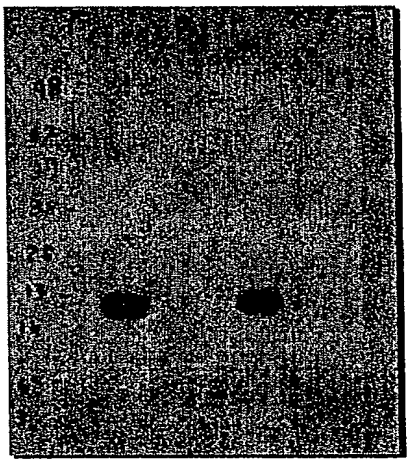


FIGURE 3

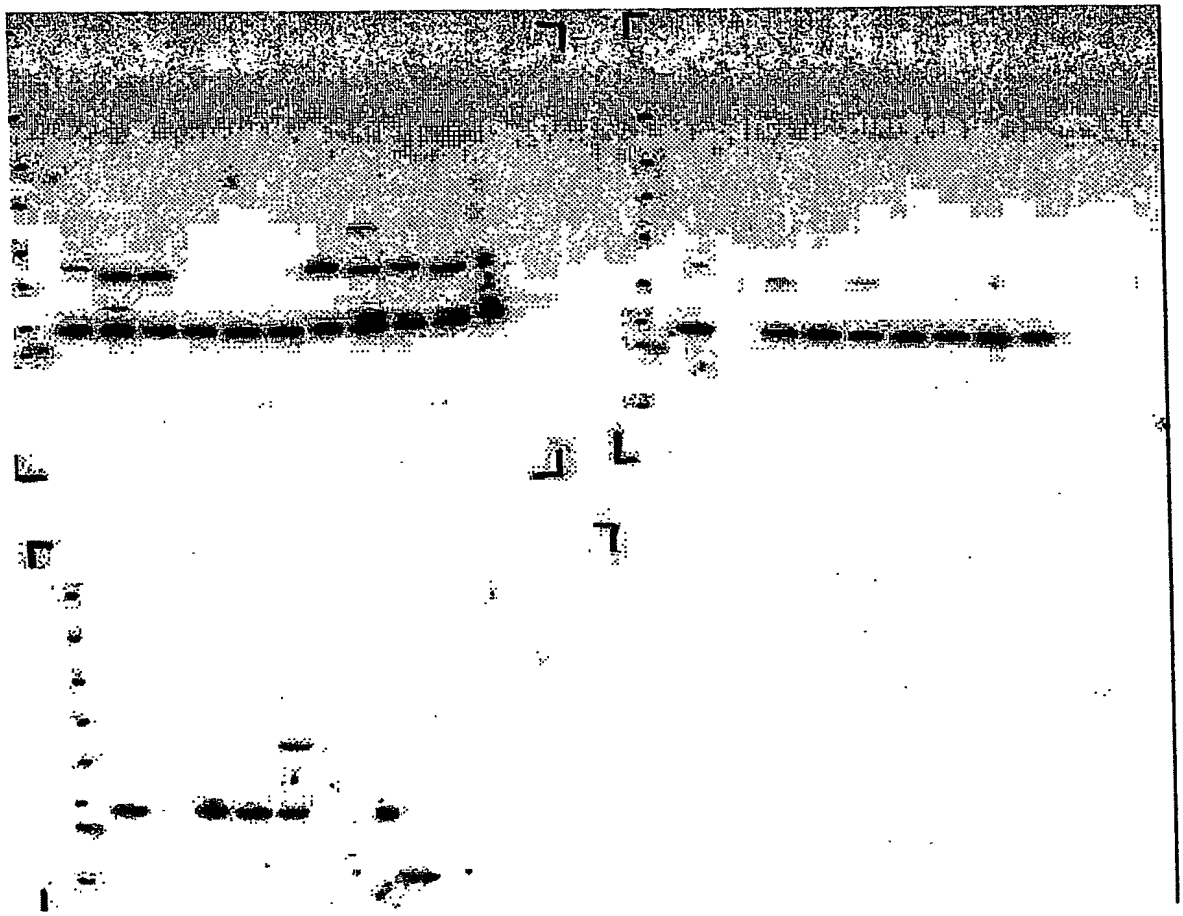


FIGURE 4

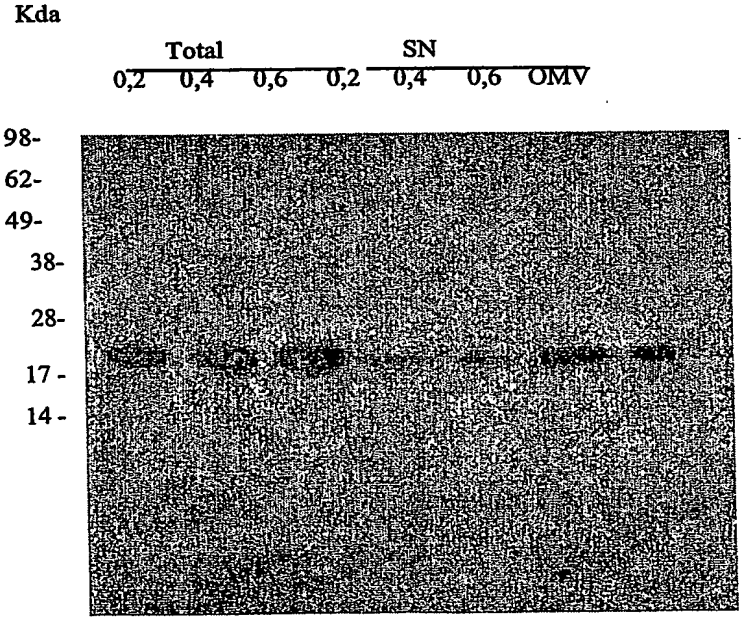


FIGURE 5

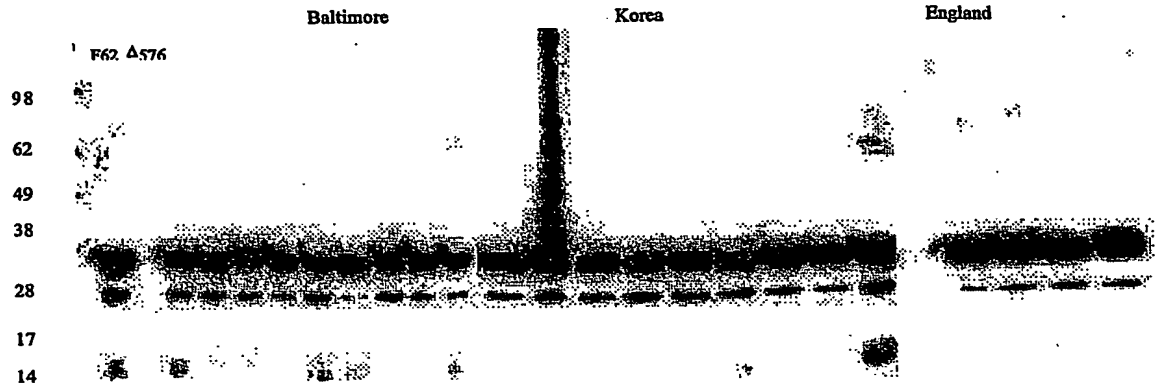
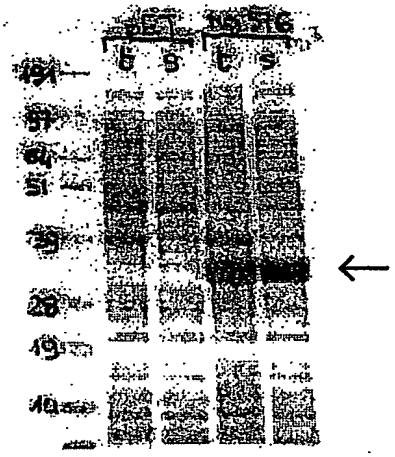


FIGURE 6



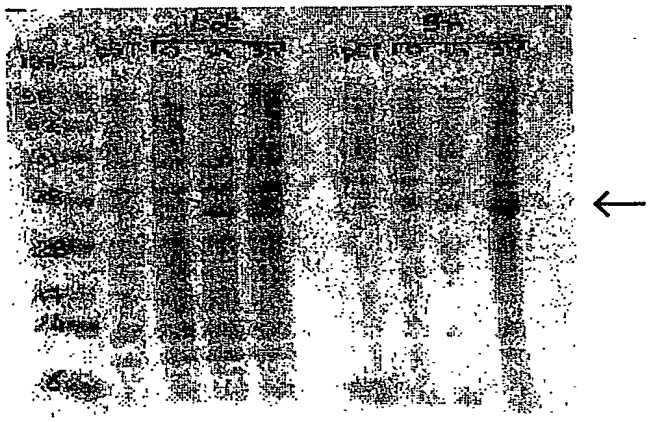


FIGURE 7

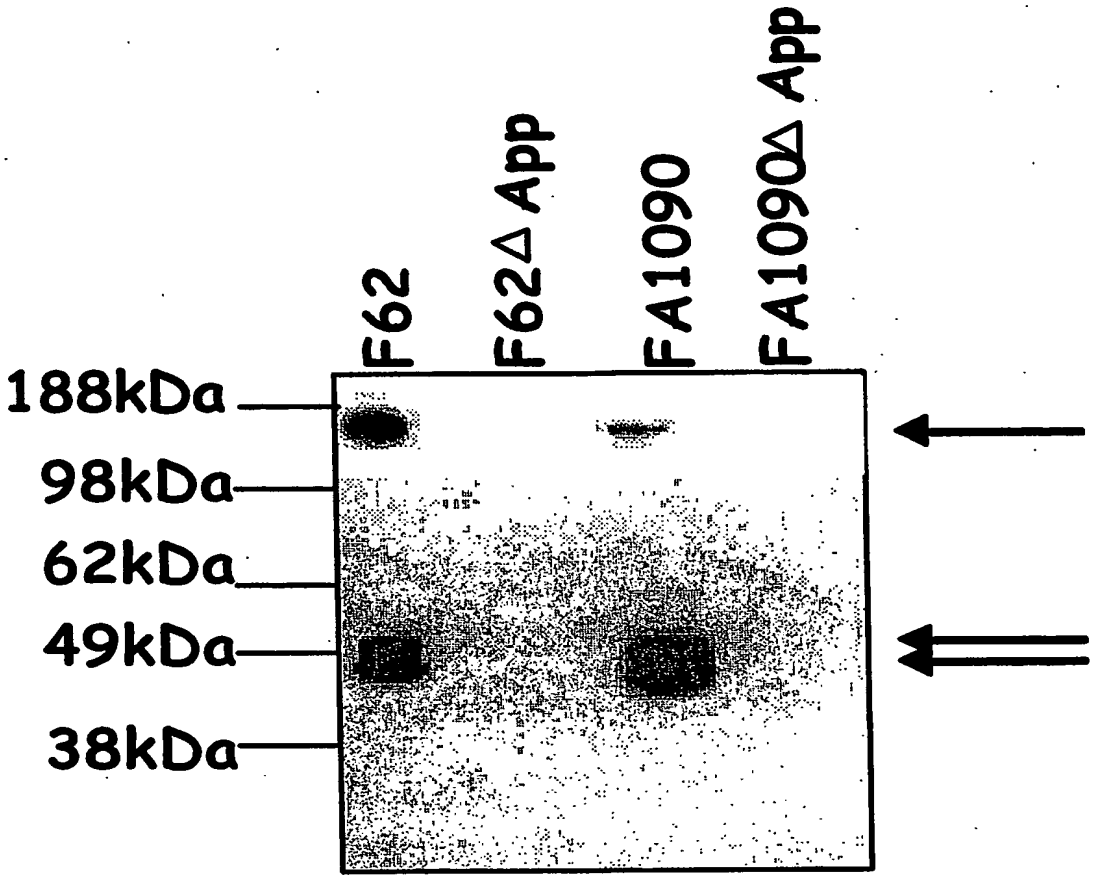


FIGURE 9

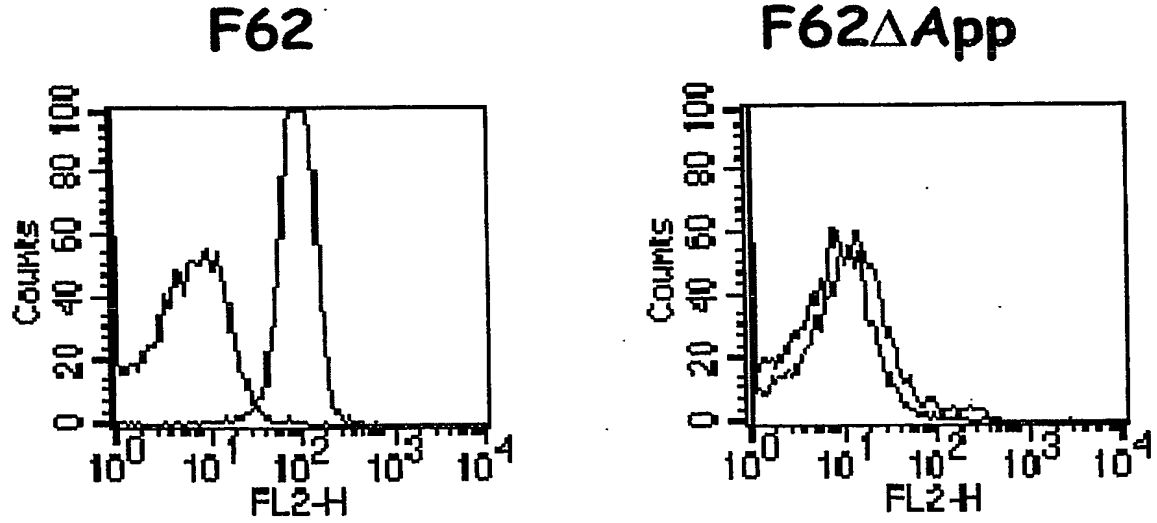


FIGURE 8

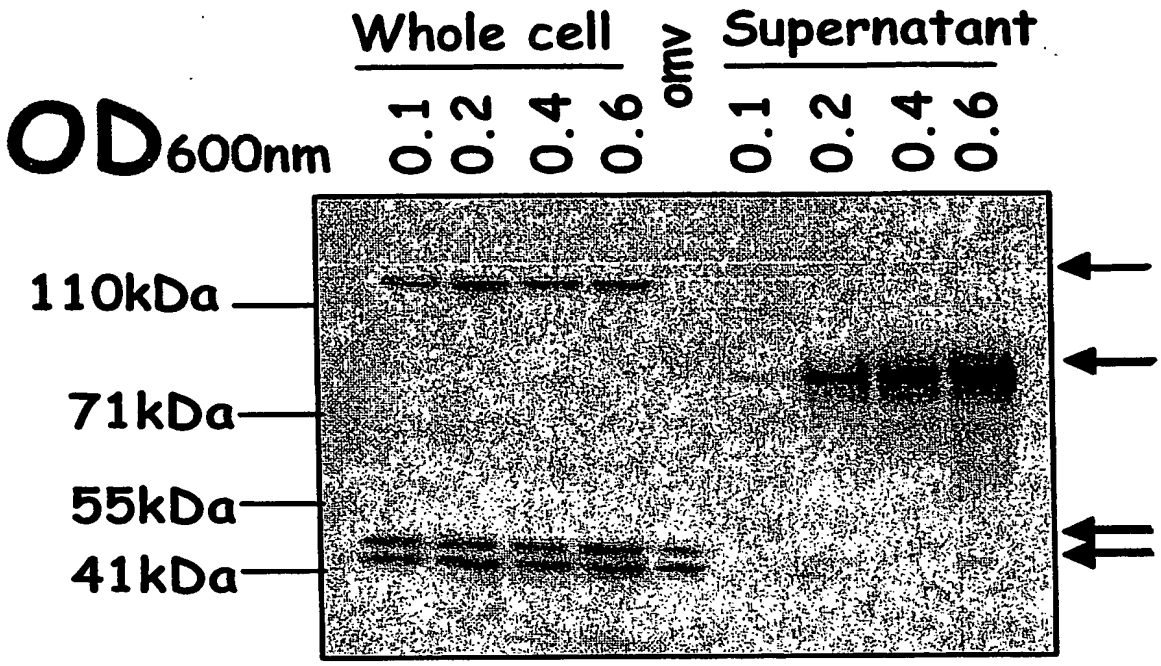
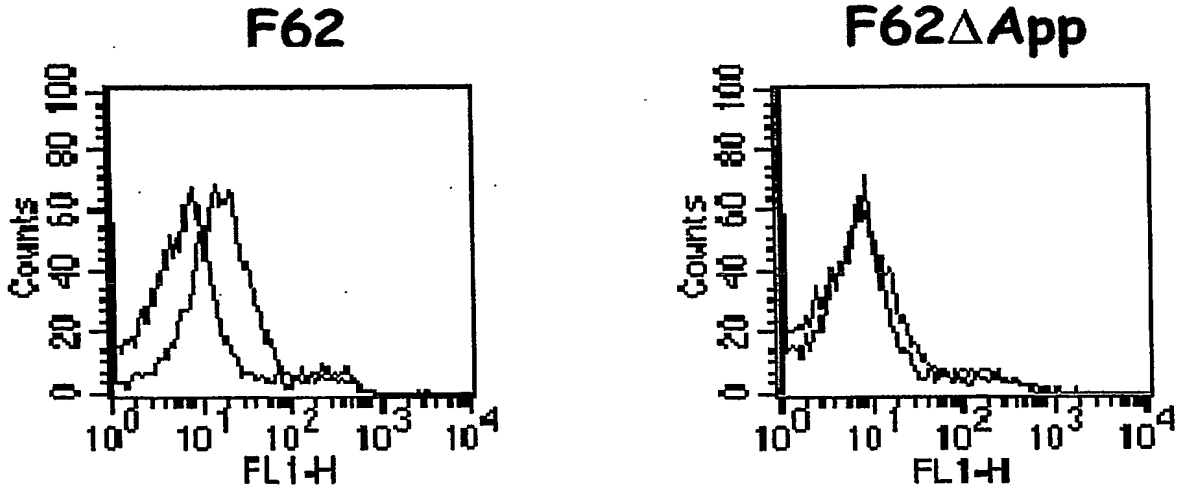


FIGURE 10



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